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[Continued on next page]

(54) Title: ULTRA HIGH AFFINITY NEUTRALIZING ANTIBODIES

A

DIQMTQSPST LSASVGDRVITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSGYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDKDYNSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNWFYFDV W GQGTTVTVSS 120
CDR H3

(57) Abstract: Ultra high affinity antibodies with binding affinities in the range of 10^{10}M^{-1} , and even 10^{11}M^{-1} are disclosed. Such antibodies include antibodies having novel high affinity complementarity determining regions (CDRs), especially those with framework and constant regions derived from either humans or mice. Methods of preparing and screening such antibodies, as well as methods of using them to prevent and/or treat disease, especially virus-induced diseases, are also disclosed.

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ULTRA HIGH AFFINITY NEUTRALIZING ANTIBODIES

5

This application claims priority of U.S. Provisional Application Serial No.
10 60/178,426, filed 27 January 2000, the disclosure of which is hereby
incorporated by reference in its entirety.

15

BACKGROUND OF THE INVENTION

The present invention relates to novel ultra high affinity neutralizing
20 antibodies.

The current incidence of infection caused by resistant or difficult to control
microbes has created a need for newer approaches to controlling such
organisms, as well as to treating those already infected.

25

Among the more difficult infectious agents to control and treat are the
viruses. For example, respiratory syncytial virus (RSV) is the major cause of
acute respiratory illness in young children admitted to hospitals and the major
cause of lower respiratory tract infection in young children. A major obstacle
30 to producing an effective vaccine against such agents as RSV has been the
issue of safety. Conversely, the use of immunoglobulins against such viral
agents has proven of some value. For example, studies have shown that
high-titred RSV immunoglobulin was effective both in prophylaxis and therapy
for RSV infections in animal models.

35

An alternative approach has been the development of antibodies, especially neutralizing monoclonal antibodies, with high specific neutralizing activity. One drawback to this route has been the need to produce human antibodies rather than those of mouse or rat and thus minimize the development of human anti-mouse or anti-rat antibody responses, which potentially results in further immune pathology.

An alternative approach has been the production of human-murine chimeric antibodies in which the genes encoding the mouse heavy and light chain variable regions have been coupled to the genes for human heavy and light chain constant regions to produce chimeric, or hybrid, antibodies.

In some cases, mouse CDRs have been grafted onto human constant and framework regions with some of the mouse framework amino acids being substituted for correspondingly positioned human amino acids to provide a "humanized" antibody. [Queen, U.S. Pat. No. 5,693,761 and 5,693,762]. However, such antibodies contain intact mouse CDR regions and have met with mixed effectiveness, producing affinities often no higher than 10^7 to 10^8 M^{-1} .

A humanized anti-RSV antibody with good affinity has been prepared and is currently being marketed. [See: Johnson, U.S. Pat. No. 5,824,307]

The production of ultra high affinity antibodies would be desirable from the point of view of both the neutralizing ability of such an antibody as well as from the more practical aspects of needing to produce less antibody in order to achieve a desirable degree of clinical effectiveness, thereby cutting costs of production and/or allowing a greater degree of clinical effectiveness for administration in the same volume.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to high affinity neutralizing antibodies and active fragments thereof exhibiting affinity constants of at least 10^{10} M^{-1} , and even 10^{11} M^{-1} , and more specifically to such a neutralizing monoclonal immunoglobulin, including antibodies and/or active fragments thereof, wherein the antibody and/or fragment has human constant regions.

The present invention solves the above-mentioned problems by providing high affinity neutralizing antibodies without the presence of intact mouse CDR regions that cause human anti-mouse antibody reactions (HAMA) and with sufficiently high affinity neutralizing activity to reduce cost and efficacy of overall production.

One aspect of the present invention relates to high affinity neutralizing antibodies with affinity constants of at least 10^{10} M^{-1} , and even 10^{11} M^{-1} , and with specificity towards specific antigenic determinants, such as those exhibited by virus-expressed proteins.

One object of the present invention is to provide such high affinity neutralizing antibodies with specificity toward antigens produced by viruses, such as where such antigens are expressed by virus-infected cells in a mammal, especially a human.

In one such embodiment, the high affinity neutralizing immunoglobulin, including antibodies and/or active fragments thereof, of the present invention, and active fragments thereof, are specific for respiratory syncytial virus (RSV), most especially for the F antigen expressed by said RSV and also expressed on the surfaces of cells infected with RSV (the presence of which antigen on the cell surface causes fusion of the cells into syncytia),

Thus, in one embodiment, a high affinity neutralizing immunoglobulin, including antibodies and/or active fragments thereof, of the present invention binds to the same epitope on RSV as the antibody whose light chain variable chain has the sequence of SEQ ID NO: 1 (shown in Figure 1A) and whose heavy chain variable chain has the sequence of SEQ ID NO: 2 (shown in Figure 1B).

It is an object of the present invention to provide ultra high affinity neutralizing antibodies having substantially the framework regions of the immunoglobulin disclosed in Figure 1 (with the same specificity of that immunoglobulin, which is an anti-RSV antibody structure) but wherein the immunoglobulins, including antibodies and active fragments thereof, of the present invention contain one or more CDRs (complementarity determining regions) whose amino acid sequences are independent of those in the so-called reference antibody, although said sequences may, in some cases, differ by no more than one amino acid and this may be limited to a difference in only one of said CDR regions.

In a preferred embodiment of the present invention, the novel immunoglobulins of the present invention will differ from the antibody of Figure 1 (hereafter, the "basic antibody" or "reference antibody" or "reference immunoglobulin") only in the sequences of one or more of the CDRs and, in a most preferred embodiment these differences occur only in CDRs L2, L3, H1, and H3.

Especially preferred embodiments of the present invention have the framework sequences depicted in Figure 1, thus having the heavy and light chain variable sequences depicted in Figures 3, 4, 5, 6, and 7.

In one embodiment, the high affinity neutralizing antibodies of the invention include a human constant region.

In a preferred embodiment, a high affinity RSV-neutralizing antibody of the invention, including active fragments thereof, with an affinity constant (K_a) of at least as high as 10^{10} M^{-1} , and even 10^{11} M^{-1} , is a recombinant immunoglobulin, such as an antibody or active fragment thereof, that includes
5 a human constant region and framework regions for the heavy and light chains wherein at least a portion of the framework is derived from a human antibody (or from a consensus sequence of a human antibody framework), an example of said framework regions depicted for the antibody sequences of Figure 1.

10 In one embodiment, all of the framework is derived from a human antibody (or a human consensus sequence).

In another highly specific embodiment, a high affinity RSV-neutralizing
15 antibody, with an affinity of at least 10^{10} M^{-1} , is a recombinant antibody having a human constant region, one or more CDRs that are derived from a non-human antibody in which at least one of the amino acids in at least one of the CDRs is changed and in which all or a portion of the framework is derived from a human antibody (or a consensus sequence of a human antibody
20 framework).

In a separate embodiment, a humanized neutralizing immunoglobulin that binds to the same epitope as the basic or reference antibody or immunoglobulin whose variable chains are shown in Figure 1, and that has an
25 affinity of at least 10^{11} M^{-1} , includes at least one of the following amino acids at the following positions of the CDRs: an alanine at position 2 of CDR H1, a phenylalanine at position 6 of CDR H3, a phenylalanine at position 3 of CDR L2, and a phenylalanine at position 5 of CDR L3. Other embodiments comprise other single amino acid substitutions at these locations.

It is another object of the present invention to provide compositions comprising the immunoglobulins disclosed herein wherein said structures are suspended in a pharmacologically acceptable diluent or excipient.

It is a still further object of the present invention to provide methods of preventing and/or treating respiratory syncytial virus comprising the administering to a patient at risk thereof, or afflicted therewith, of a therapeutically effective amount of a composition containing an immunoglobulin of the invention, such as where said antibodies or active fragments thereof exhibit the specificity and affinity properties disclosed herein for the immunoglobulins of the invention.

DEFINITIONS

The term "antigen" refers to a structure, often a polypeptide or protein, present on the surface of a microorganism, such as a virus, for which an antibody has affinity and specificity.

The term "antigenic determinant" refers to a specific binding site on an antigenic structure for which an immunoglobulin, such as an antibody, has specificity and affinity. Thus, a particle, such as a virus, may represent an antigen but may have on its surface a number of separate, and different, antigenic sites such as where the virus has a number of different surface proteins and each represents a distinct potential binding site for an immunoglobulin.

The term "immunoglobulin" refers to a protein or polypeptide having specificity and affinity for an antigen or antigenic determinant. This term includes antibodies, commonly depicted as tetrameric, as well as active fragments thereof, such fragments having specificity and affinity for antigens

or antigenic determinants. Thus, "immunoglobulin" as used herein includes antibodies and all active fragments thereof.

5 The term "antibody" refers to a protein or polypeptide having affinity for an antigenic determinant, usually one found on the surface of a microorganism, especially a virus. Such an antibody is commonly composed of 4 chains and is thus tetrameric.

10 The term "neutralizing immunoglobulin" or "neutralizing antibody" refers to the ability of the immunoglobulins, including antibodies, of the present invention to reduce the replication of microorganisms, especially viruses, in organisms as well as in cell cultures. An indication of such ability is the data from the microneutralization assays disclosed hereinbelow. Such a structure
15 usually has both variable and constant regions whereby the variable regions are mostly responsible for determining the specificity of the antibody and will comprise complementarity determining regions (CDRs).

20 The term "complementarity determining region" or "CDR" refers to variable regions of either H (heavy) or L (light) chains contains the amino acid sequences capable of specifically binding to antigenic targets. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure. Such regions are also referred to as
25 "hypervariable regions."

30 The term "active fragment" refers to a portion of an antibody that by itself has high affinity for an antigenic determinant and contains one or more CDRs accounting for such specificity. Non-limiting examples include Fab, F(ab)₂, heavy-light chain dimers, and single chain structures, such as a complete light chain or complete heavy chain.

The term "specificity" refers to the ability of an antibody to bind preferentially to one antigenic site versus a different antigenic site and does not necessarily imply high affinity.

5 The term "affinity" refers to the degree to which an antibody binds to an antigen so as to shift the equilibrium of antigen and antibody toward the presence of a complex formed by their binding. Thus, where an antigen and antibody are combined in relatively equal concentration, an antibody of high affinity will bind to the available antigen so as to shift the equilibrium toward
10 high concentration of the resulting complex.

 The term "affinity constant" refers to an association constant used to measure the affinity of an antibody for an antigen. The higher the affinity constant the greater the affinity of the immunoglobulin for the antigen or
15 antigenic determinant and vice versa. An affinity constant is a binding constant in units of reciprocal molarity. Such a constant is readily calculated from the rate constants for the association-dissociation reactions as measured by standard kinetic methodology for antibody reactions.

20

BRIEF DESCRIPTION OF THE DRAWINGS

 Figure 1 shows the amino acid sequence of the light and heavy
25 chain variable regions of an anti-RSV antibody wherein the CDR regions are underlined while non-underlined residues form the framework regions of the variable regions of each chain. In this antibody, the CDRs are derived from a mouse anti-RSV antibody while the framework regions consist mostly of sequences derived from a human antibody. For each CDR, locations at which
30 amino acid replacements were used to achieve the high affinity CDRs and antibodies disclosed herein are in bold face. In accordance with the disclosure herein, such replacements were only in CDRs L2, L3, H1 and H3. Figure 1A

shows the light chain variable region and Figure 1B shows the heavy chain variable region of the light and heavy chains, respectively. Constant region sequences are not shown. These sequences are present in the basic clone (see Table 2), designated IX-493 throughout this disclosure (i.e., SWSG - meaning a serine (S) at the key position (see tables 1 and 3) of high affinity CDR H1, a tryptophan (W) at the key position of high affinity CDR H3, a serine (S) at the key position of high affinity CDR L2, and a glycine (G) at the key position of high affinity CDR L3). For purposes of this disclosure, this is the "reference antibody."

Figure 2 shows affinity comparisons for a particular set of beneficial or high affinity clones. The clonal designations are on the left of the legend at the right of the drawing along with the indicated substitutions at CDRs H1, H3, L2, and L3 shown on the right of the legend. Measurements are by ELISA (OD at 560 nm shown on the left axis). Clone L1FR represents the results for the reference antibody structure of Figure 1.

Figure 3 shows the heavy and light chain variable regions for the preferred embodiment of clone 1 (Table 2) of the invention disclosed herein. CDR regions are underlined while the amino acid differences versus the antibody of Figure 1 are indicated in bold face. Thus, this preferred (i.e., high affinity) antibody has several of the high affinity CDRs (Table 3) present which give rise to higher affinity (over 10^{10} M^{-1}) than the basic or reference antibody.

Figure 4 shows the heavy and light chain variable regions for the preferred embodiment of clone 2 (Table 2) of the invention disclosed herein. CDR regions are underlined while the amino acid differences versus the antibody of Figure 1 are indicated in bold face. Thus, this preferred (i.e., high affinity) antibody has several of the high affinity CDRs (Table 3) present which give rise to higher affinity (over 10^{10} M^{-1}) than the basic or reference antibody.

Figure 5 shows the heavy and light chain variable regions for the preferred embodiment of clone 3 (Table 2) of the invention disclosed herein. CDR regions are underlined while the amino acid differences versus the antibody of Figure 1 are indicated in bold face. Thus, this preferred (i.e., high
5 affinity) antibody has several of the high affinity CDRs (Table 3) present which give rise to higher affinity (over 10^{10} M^{-1}) than the basic or reference antibody.

Figure 6 shows the heavy and light chain variable regions for the most preferred embodiment of clone 22 (Table 4) of the invention disclosed herein.
10 CDR regions are underlined while the amino acid changes versus the antibody of Figure 1 are indicated in bold face. Thus, this most preferred (i.e., highest affinity) antibody has several of the high affinity CDRs (Table 3) present which give rise to higher affinity (over 10^{11} M^{-1}) than the basic or reference antibody).

15 Figure 7 shows the heavy and light chain variable regions for the preferred embodiment of clone 23 (Table 4) of the invention disclosed herein. CDR regions are underlined while the amino acid changes versus the antibody of Figure 1 are indicated in bold face. Thus, this preferred (i.e.,
20 highest affinity) antibody, has several of the high affinity CDRs (Table 3) present which give rise to higher affinity (over 10^{11} M^{-1}) than the basic or reference antibody).

Figure 8 shows the results of microneutralization experiments on
25 several of the ultra-high affinity antibody clones disclosed herein. The amino acids present at the key positions of the high affinity complementarity determining regions (see Table 3) are shown at the right in the order H1, H3, L2, and L3 (as also shown in Table 2 where the clones are simply numbered but the table compares to this figure by relying on the actual amino acids used
30 at the critical positions as disclosed in the Table and in the legend at the right of this figure). Thus, for clone 4D2-7, there is an alanine (A) at the key position of high affinity CDR H1, a phenylalanine (F) at the key position of

high affinity CDR H3, a phenylalanine (F) at the key position of high affinity CDR L2, and a phenylalanine (F) at the key position of high affinity CDR L3. Briefly, about 25,000 HEp-2 cells were added to each of the wells of a 96 well plate along with RSV and a given concentration of the antibody (antibody concentration per well is shown on the abscissa – See Example 2 for exact details). After 5 days growth, the cells were fixed, treated with biotinylated anti-F MAb, then bound to avidin-peroxidase complex and the ability of the bound peroxidase to react thionitrobenzoic acid was determined by measuring O.D. at 450 nm. The amount of F protein present was an indicator of the extent of viral replication thereby resulting in more reaction of substrate by peroxidase and increased absorption. Thus, the lower the OD 450 value, the greater the neutralizing ability of the indicated concentration of the given antibody. Here, IX-493 (SWSG - meaning a serine (S) at the key position of high affinity CDR H1, a tryptophan (W) at the key position of high affinity CDR H3, a serine (S) at the key position of high affinity CDR L2, and a glycine (G) at the key position of high affinity CDR L3) is the "reference antibody" (also denoted L1FR and IX493L1FR).

Figure 9 shows results for microneutralization assays of several of the antibodies disclosed herein but where only Fab fragments were employed for neutralization of antibody replication. Here, IX-493 (SWSG) is the reference Fab fragment and is derived from antibody Medi-493 (see: Johnson et al (1997) – ref. 23).

Figure 10 shows the results of microneutralization for an antibody specific for RSV as compared to similar experiments for Fab fragments of the same antibody. Here, Medi 493 represents the antibody while IX-493 LIFR represents the Fab fragment of this antibody. The other lines are for Fab fragments of several of the antibodies produced according to the present invention (given various letter-digit code designations for internal identification but having nothing to do with their relative efficacy as neutralizing antibodies).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to ultra high affinity neutralizing antibodies and active fragments thereof having affinity constants of at least 10^{10} M^{-1} . Active fragments of these antibodies are fragments containing at least one high affinity complementarity determining region (CDR).

With the advent of methods of molecular biology and recombinant technology, it is now possible to produce antibody molecules by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with *in vitro* assembly of the synthesized chains to form active tetrameric (H_2L_2) structures with affinity for specific epitopes and antigenic determinants. This has permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

Regardless of the source of the immunoglobulins, or how they are recombinantly constructed, or how they are synthesized, *in vitro* or *in vivo*, using transgenic animals, such as cows, goats and sheep, using large cell cultures of laboratory or commercial size, in bioreactors or by direct chemical synthesis employing no living organisms at any stage of the process, all immunoglobulins have a similar overall 3 dimensional structure. In the case of an antibody, this structure is often given as H_2L_2 and refers to the fact that antibodies commonly comprise 2 light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity.

The variable region of either H or L chains contains the amino acid sequences capable of specifically binding to antigenic targets. Within these sequences are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies or active fragments of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure.

The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all canonical antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light (L) and heavy (H) chains. The accepted CDR regions have been described by Kabat et al, *J. Biol. Chem.* **252**:6609-6616 (1977). The numbering scheme is shown in the figures, where the CDRs are underlined and the numbers follow the Kabat scheme.

In all mammalian species, antibody polypeptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

The immunoglobulins disclosed according to the present invention afford extremely high affinity (on the order of 10^{10} M^{-1} , and even 10^{11} M^{-1} , for the affinity constant, or K_a , defined as an association constant describing the binding of antibody and antigen as the ligands) for epitopes, or antigenic determinants, found in macromolecules, especially proteins, and most especially proteins expressed by viruses and other microorganisms, such as

proteins expressed on the surfaces of viruses as well as the surfaces of cells infected with a virus. The high affinity antibodies of the present invention are neutralizing antibodies and thus reduce the replication of viruses in organisms as well as in cell cultures while maintaining sufficient homology to the antibody amino acid sequences of the recipient so as to prevent adverse immunological pathology. The latter feature is achieved through the use of constant regions similar to those of the recipient organism, such as a mammal and most especially a human. This feature is also achieved through the use of framework amino acid sequences similar, if not identical, to those found in antibodies from the recipient organism. In the latter case, some amino acid replacements may be made in the framework sequences so as to facilitate and maintain the high affinity interaction between the novel CDRs of the present invention and the antigen for which said antibodies show specificity.

As used herein, terms such as "antibody" and "active fragment" or "fragment" are not to be considered limiting in determining the full extent of the present invention. Thus, the fact that the term "antibody" is used rather than "active fragment" or "immunoglobulin" is not to be taken as a limitation on the invention or its uses so long as the requisite properties of specificity and affinity are exhibited by said structure.

In accordance with the invention disclosed herein, the affinity constants characterizing the affinities of the high affinity neutralizing antibodies, and active fragments thereof, of the present invention are association constants and were measured by the kinetics of antigen-antibody complex formation, with the rate constant for association to form the complex being denoted as k_{assoc} or k_{on} and the dissociation constant being denoted as k_{diss} or k_{off} . Measurement of such constants is well within the ordinary skill in the art and the details will not be described further herein except for general methodology and specific conditions, where appropriate, as recited in the examples given herein to further describe the invention.

The high affinity antibodies of the present invention can be achieved, as already described, through genetically engineering appropriate antibody gene sequences, i.e., amino acid sequences, by arranging the appropriate nucleotide sequences and expressing these in a suitable cell line. Any desired
5 nucleotide sequences can be produced using the method of codon based mutagenesis, as described, for example, in U.S. Pat. Nos. 5,264,563 and 5,523,388. Such procedures permit the production of any and all frequencies of amino acid residues at any desired codon positions within an oligonucleotide. This can include completely random substitutions of any of
10 the 20 amino acids at an desired position or in any specific subset of these. Alternatively, this process can be carried out so as to achieve a particular amino acid a desired location within an amino acid chain, such as the novel CDR sequences according to the invention. In sum, the appropriate nucleotide sequence to express any amino acid sequence desired can be
15 readily achieved and using such procedures the novel CDR sequences of the present invention can be reproduced. This results in the ability to synthesize polypeptides, such as antibodies, with any desired amino acid sequences. For example, it is now possible to determine the amino acid sequences of any desired domains of an antibody of choice and, optionally, to prepare
20 homologous chains with one or more amino acids replaced by other desired amino acids, so as to give a range of substituted analogs.

In applying such methods, it is to be appreciated that due to the degeneracy of the genetic code, such methods as random oligonucleotide
25 synthesis and partial degenerate oligonucleotide synthesis will incorporate redundancies for codons specifying a particular amino acid residue at a particular position, although such methods can be used to provide a master set of all possible amino acid sequences and screen these for optimal function as antibody structures or for other purposes. Such methods are described in
30 Cwirla et al, *Proc. Natl. Acad. Sci.* **87**:6378-6382 (1990) and Devlin et al., *Science* **249**:404-406 (1990).

In accordance with the invention disclosed herein, enhanced antibody variants can be generated by combining in a single polypeptide structure one, two or more novel CDR sequences according to the invention, each shown to independently result in enhanced binding activity. In this manner, several novel amino acids sequences can be combined into one antibody, in the same or different CDRs, to produce high affinity antibodies within the present invention. For example, 3 such novel CDR sequences may be employed and the resulting antibodies screened for affinity for a particular antigenic structure, such as the F antigen or RSV. The overall result would thus be an iterative process of combining various single amino acid substitutions and screening the resulting antibodies for antigenic affinity in a step by step manner. Such methods were used to prepare some of the antibodies embodied within the present invention. Such methods also represent a convenient, if tedious, means of optimizing the antibody sequences of the present invention, especially the sequences of the CDR regions of said antibodies.

Using the novel sequences and methods of the present invention, such an approach avoids the time and expense of generating and screening all possible permutations and combinations of antibody structure in an effort to find the antibody with the maximum efficiency. Conversely, complete randomization of a single 10 amino acid residue CDR would generate over 10 trillion variants, a number virtually impossible to screen.

This iterative method can be used to generate double and triple amino acid replacements in a stepwise process so as to narrow the search for antibodies having higher affinity.

Conversely, it should be recognized that not all locations within the sequences of the different antibody domains may be equal. Substitutions of any kind in a particular location may be helpful or detrimental. In addition, substitutions of certain kinds of amino acids at certain locations may likewise

be a plus or a minus as it affects affinity for the particular antigen. For example, it may not be necessary to try all possible hydrophobic amino acids at a given position. It may be that any hydrophobic amino acid will do as well. Alternatively, an acidic or basic amino acid at a given location may provide
5 large swings in measured affinity. It is therefore necessary also to learn the "rules" of making such substitutions but the determination of such "rules" may not require the study of all possible combinations and substitutions – trends may become apparent after examining fewer than the maximum number of substitutions.

10

In accordance with the foregoing, the antibodies of the present invention are ultra high affinity neutralizing antibodies, such as anti-RSV antibodies, and in the latter case most preferably antibodies with specificity toward the same epitope of RSV as the antibody of U.S. Patent No.
15 5,824,307.

In addition, the affinities of the ultra high affinity antibodies of the invention typically are at least 10^{10} M^{-1} . Because such high affinities are not easily measured, except by the procedures described herein, such value may
20 commonly be considered as part of a range and may, for example, be within 2 fold of 10^{10} M^{-1} or be greater than 10^{10} M^{-1} or may even be numerically equal to 10^{10} M^{-1} . In such cases, the affinity is denoted by an affinity constant, which is in the nature of a binding constant so as to give units of reciprocal molarity. As such, the affinity of the antibody for antigen is proportional to the value of
25 this constant (i.e., the higher the constant, the greater the affinity). Such a constant is readily calculated from the rate constants for the association-dissociation reactions as measured by standard kinetic methodology for antibody reactions.

30 In a specific embodiment, the high affinity neutralizing antibodies of the present invention, and active fragments thereof, have affinity constants for

their respective antigens of at least at least 10^{11} M^{-1} , in some cases being in excess of this value, a range at the very upper region of measurability.

The high affinity neutralizing antibodies of the present invention, and
5 active fragments thereof, may be advantageously directed to any antigenic determinants desired, such as epitopes present on any type of macromolecule, especially peptide epitopes present as part of the three dimensional structure of protein and polypeptide molecules. Such peptide epitopes may commonly be present on the surfaces, or otherwise be part of
10 the structure of, microorganisms and cells, such as cancer cells. Microorganisms expressing such peptide epitopes includes bacteria and viruses. In the latter case, the proteins and polypeptides exhibiting peptide epitopes may be molecules expressed on the surfaces of the virus or may be expressed by cells infected with a virus. For purposes of evaluating the
15 efficacy of the rules and methods disclosed according to the present invention, a virus was chosen as one available antigenic source for developing antibodies within the present invention. The virus chosen for further analysis was the respiratory syncytial virus (RSV). This latter virus was chosen because it is well characterized with respect to its replicative cycle as
20 well as with respect to the antigenic determinants found on its surface. In addition, antigens known to be expressed by cells infected with this virus are well characterized. Thus, the virus exhibits both a surface G antigen and a surface F antigen, both proteins. The G antigen facilitates binding of the virus to cell surfaces and the F proteins facilitates the fusion of the virus with cells.
25 The cells so infected also express the F antigen on their surfaces and the latter result induces fusion of the cells to form a syncytium, hence the name of the virus. In addition, this virus is a convenient subject for analysis in that cell lines readily infected by this virus are well known and well characterized, thereby making it an easy virus to grow and culture *in vitro*. Further,
30 antibodies available for treatment of this virus are known and are commercially available (for example, the antibodies disclosed in U.S. Patent No. 5,824,307). For these reasons, the antibody disclosed in said patent, the

disclosure of which patent is hereby incorporated by reference in its entirety, contains the amino acid sequences of the "reference antibody" disclosed in Figure 1 and whose CDR sequences are summarized in Table 1. Thus, the availability of a commercially successful antibody product as well as the well characterized properties of RSV made this an ideal combination to use in testing and optimizing the antibodies of the present invention and the rules disclosed herein for producing high affinity CDRs for use in constructing such antibodies. Otherwise, the antibodies disclosed herein, as well as the methods disclosed herein for preparing such antibodies, are in no way limited to RSV as the antigen but are readily and advantageously applied generally to the field of antibody technology. In addition, even the specific embodiments provided by the present disclosure and which are directed specifically to antigenic determinants expressed by RSV, and cells infected with RSV, also may have high affinity for other epitopes, especially those present on related viruses. Therefore, as disclosed in accordance with the present invention, RSV, and the antibody with variable sequences as shown in Figure 1, are merely a convenient model system used as a reference point for developing and applying the methods of antibody technology taught by the present invention.

A high affinity neutralizing antibody according to the present invention, including active fragments thereof, comprises at least one high affinity complementarity determining region (CDR) wherein said CDR has an amino acid sequence selected to result in an antibody having an affinity constant (K_a) of at least $10^{10}M^{-1}$. In preferred embodiments, such antibody, or active fragment, comprises at least 2 high affinity CDRs, or at least 3 high affinity CDRs or even at least 4 high affinity CDRs. In highly preferred embodiments, such antibodies or active fragments comprise 3 or 4 high affinity CDRs. In one preferred embodiment, such active fragment is an Fab fragment.

The high affinity antibodies of the present invention commonly comprise a mammalian, preferably a human, constant region and a variable

region, said variable region comprising heavy and light chain framework regions and heavy and light chain CDRs, wherein the heavy and light chain framework regions are derived from a mammalian antibody, preferably a human antibody, and wherein the CDRs are derived from an antibody of some species other than a human, preferably a mouse. Where the framework amino acids are also derived from a non-human, the latter is preferably a mouse.

In addition, high affinity antibodies of the invention bind the same epitope as the antibody from which the CDRs are derived, and wherein at least one of the CDRs of said ultra high affinity antibody contains amino acid substitutions, and wherein said substitutions comprise the replacement of one or more amino acids in the CDR regions by non-identical amino acids, preferably the amino acids of the correspondingly aligned positions of the CDR regions of the human antibody contributing the framework and constant domains.

The high affinity CDRs may be produced by amino acid substitutions in non-high affinity CDRs to produce such high affinity CDRs or such high affinity CDRs may be synthesized directly to form such high affinity CDRs. Thus, the ultra high affinity neutralizing antibodies of the present invention may have amino acid substitutions in only one of the CDR regions, preferably more than one CDR region, and most preferably 3 or even 4 such regions, with possibly as many as 5 or even all 6 of the CDRs containing at least one substituted amino acid.

In applying the methods disclosed herein to produce antibodies of the present invention, the method of preparing the antibodies is not a limiting factor. Thus, the high affinity neutralizing antibodies of the present invention may be prepared by generating polynucleotide sequences coding for the polypeptides of the antibodies and using vectors to insert said polynucleotide sequences into permissive cells capable of not only expressing such polypeptides but also of assembling them into characteristic tetrameric

antibody structures that are then retrieved from the cells or cell cultures, possibly being secreted into the medium by such cells. Technologies for such manufacturing procedures are already known and patented and are not essential to practicing the present invention. [see: Morrison et al, U.S. Patent
5 No. 5,807,715]. In addition, the polypeptide chains of the antibodies of the present invention may be synthesized chemically, with or without the addition of enzymes, and then chemically joined to form tetrameric structures of the usual H₂L₂ configuration. Thus, any method of preparing the antibodies disclosed herein can be utilized.

10

The present invention is also directed to the formation of high affinity neutralizing antibodies, with the properties already enumerated, having high affinity as a result predominantly of having high affinity CDR sequences. In accordance with the present invention, the CDR sequences of the antibodies
15 disclosed herein have been optimized so as to confer upon the antibody molecule the ultra high affinities characteristic of the antibodies of the present invention. Such CDR sequences, together with the framework sequences disclosed herein, especially those taught by the sequences of Figures 1, 3, 4, 5, 6, and 7, and most especially when used with constant region sequences
20 characteristic of the antibodies of the organism acting as recipient of the antibodies of the present invention when used therapeutically, produce the antibodies of the present invention in their more specific embodiments. However, the methods of the present invention are more specifically directed to the CDR amino acid sequences.

25

To produce immunoglobulins, such as antibodies and/or active fragments thereof, within the present invention, e.g., high affinity neutralizing antibodies, the rules taught by the present invention are used advantageously to produce antibody molecules whose structures incorporate the sequences of
30 the high affinity CDR sequences disclosed according to the present invention. Thus, the high affinity neutralizing antibodies of the present invention are, in essence, not truly "monoclonal" antibodies as that term is commonly used,

since they do not have to be produced by cloning anything. As already mentioned, the sequences of the antibodies of the invention may be directly synthesized and thus may not be identical to any antibody sequences, especially not to any CDR sequences, presently known. The sequences themselves can be wholly novel *ab initio* and not be exactly represented in any antibody produced by any living organism. Thus, the high affinity CDR sequences disclosed herein are found, or achieved, by optimization, as disclosed herein, and then, once said high affinity CDR sequences are known, can be used to synthesize fully functional antibody molecules, whether dimeric or tetrameric, bifunctional or monofunctional, by any and all means known to science.

In keeping with the foregoing, and in order to better describe the sequences disclosed according to the invention, including their optimization, the sequence of the light and heavy chain variable regions of a reference antibody (here, the anti-RSV antibody of U.S. Patent No. 5, 5,824,307) are shown in Figure 1A (light chain variable region – SEQ ID NO: 1) and Figure 1B (heavy chain variable region – SEQ ID NO: 2). Also in accordance with the invention, novel sequences were produced with amino acid differences only in CDR regions relative to the reference antibody. One means utilized to accomplish this result was to introduce mutations in CDR regions of the so-called starting or reference chains and then assay the resulting recombinatorial clones for antigen (RSV F protein) affinity.

In accordance with the foregoing, changes were made in first one CDR sequence to optimize that sequence and determine the "critical" residue, or residues, than said position(s) was optimized through a series of amino acid substitutions limited to that position alone. Each of the 6 CDRs of the antibody clone was studied in turn until the "critical" CDRs were determined (wherein "critical CDRs" means CDRs having a substantial effect on antibody binding, such as the beneficial or high affinity CDRs of Table 3). No all CDRs were found to be critical. For the antibody used in this particular study, only CDRs

H1, H3, L2, and L3 were found to be critical but the results may be different for a different antibody. Once a "high affinity" CDR was determined (i.e., a "beneficial CDR") then combinations of the CDRs were studied to optimize the combination of CDR sequences resulting in a high affinity neutralizing antibody of the invention.

As a very specific embodiment, the invention disclosed herein relates to a high affinity neutralizing antibody against respiratory syncytial virus (RSV) having an affinity constant of at least 10^{10} M^{-1} , wherein said affinity constant could be within at least 2 fold of this value because of the variability of such determinations and the variability of affinity of the different cloned antibodies for the antigen (here, F antigen of RSV). Some of the resulting optimized structures within this embodiment had K_a greater than 10^{11} M^{-1} (for example, clones numbered 22 and 23 in Table 4).

This high affinity neutralizing antibody is also an antibody that binds to the same epitope on RSV as the antibody whose light chain variable region has the sequence of SEQ ID NO: 1 (Figure 1A) and whose heavy chain variable region has the sequence of SEQ ID NO: 2 (Figure 1B).

In general, the approach used to identify antibodies of the invention, based on the specific example of anti-RSV just described, was to generate nucleotide sequences for the genes expressing the desired antibody chains and insert these into vectors then used to transform *Escherichia coli* cells by standard protocols. The cells were grown in wells and the supernatant sampled and measured for antigen binding using capture lift and ELISA techniques. [See: Watkins et al, (1997) *Anal. Biochem.* **253**, 37-45; Watkins et al, (1998) *Anal. Biochem.* **256**, 169-177 (the specifications of which are incorporated herein by reference)] These polynucleotides were designed so as to provide single amino acid replacements in the CDRs that could then be screened for increased affinity, with beneficial replacements (those yielding increased affinity) being selectively combined for increased affinity. These

were then screened for binding affinity for F antigen of RSV versus the basic or reference antibody.

Using this protocol, ELISA data indicated that no single amino acid
5 replacements in CDRs L1 or H2 produced any increase in the affinity of the
antibody clone for the epitope used as antigen (here, the F antigen of RSV).
Therefore, the antibodies of the present invention all contain CDR sequences
that differ from the reference antibody only in CDRs L2, L3, H1 and H3 (here,
the reference antibody with sequences in Figure 1 was merely a useful
10 reference against which to test procedures for optimization of antibody affinity
by increasing K_a and any other system could be used equally well).

The antibodies thus disclosed herein with respect to RSV also
commonly have framework regions derived from a human antibody but, where
15 not so derived, preferably from a mouse.

For the CDRs of the reference antibody, the amino acid sequence of
each CDR (as given in the sequences of Figure 1) is shown in Table 1. Amino
acid residue locations within the CDRs of the basic or reference antibody,
20 which, if replaced by amino acids as taught by the present invention, following
optimization, produced high affinity CDR sequences (resulting in very high
affinity neutralizing antibodies) and thereby a beneficial result (increase in
affinity) are indicated in bold face and underlining in Table 1 (such sequences
giving increased affinity over the reference antibody being denoted as
25 "beneficial CDRs" or "high affinity CDRs"). The CDRs of the basic or
reference antibody (Figure 1) are referred to herein as "basic or reference
CDRs"). Thus, Table 1 represents the CDR sequences depicted in Figure 1
(i.e., for the anti-RSV reference antibody used herein to monitor optimization).

30

Table 1. Sequences of Basic or Reference CDRs

	<u>CDR</u>	<u>Length</u>	<u>Sequence</u>	<u>SEQ ID NO.</u>
5	L1	10	S A S S S V G Y M H	3
	L2	7	D T <u>S</u> K L A S	4
	L3	9	F Q G S <u>G</u> Y P F T	5
	H1	7	T <u>S</u> G M S V G	6
	H2	16	D I W W D D K K D Y N P S L K S	7
10	H3	10	S M I T N <u>W</u> Y F D V	8

With respect to the sequences disclosed herein, the CDR regions as defined for purposes of the present invention are those segments corresponding to residues 24-33 (CDR L1), 49-55 (CDR L2) and 88-95 (CDR L3) of the light chain variable regions and residues 31-37 (CDR H1), 52-67 (CDR H2) and 100-109 (CDR H3) of the heavy chain variable regions of the antibodies disclosed herein.

In producing the antibodies of the present invention, whether by generating clones or cloning the polypeptide chains composing said antibodies, or by direct synthesis of the polypeptide sequences, with or without the use of polynucleotide sequences coding therefor, or by whatever method the user may choose, since no method of producing said antibodies results in a limitation of the teaching of the present invention, the basic or reference antibody (heavy and light chain variable regions (CDRs plus Framework) shown in Figure 1) can be used as a "template" for generating the novel CDR sequences of the antibodies of the present invention and for purposes of comparing binding constants, etc. Standard approaches to characterizing and synthesizing the six CDR libraries of single mutations were used (see Wu et al, *Proc. Natl. Acad. Sci.* **95**:6037-6042 (1998)). The target CDR was first deleted for each of the libraries prior to annealing the nucleotides. For synthesis of the libraries, the CDRs were defined as in Table

1. Codon based mutagenesis for oligonucleotide synthesis to yield the CDR sequences of the invention was employed (as described above).

5 Libraries were initially screened by capture lift to identify the highest affinity variants. Subsequently, these clones were further characterized using capture ELISA and by titration on immobilized antigen.

10 DNA from the highest affinity variants was sequenced to determine the nature of the beneficial or high affinity replacements. After screening, it was determined that eight beneficial or high affinity replacements, occurring in only four of the CDRs, had been observed. These are summarized as the CDR sequences in Table 3 with differences versus the reference or basic CDRs of Table 1 being bold and underlined. Thus, the CDR sequences of Table 3 can be considered a CDR library of cassettes available for use in producing a high
15 affinity neutralizing antibody of the present invention where specificity is directed toward the F antigen of RSV.

20 Analysis of the data indicated that replacement of amino acids at selected locations had greatly increased epitope binding, especially where the nature of the replacement was the insertion of an amino acid selected from the group phenylalanine, alanine, proline, tryptophan and tyrosine, most especially phenylalanine, all such beneficial or high affinity replacements again being at selected positions.

25 For the optimization experiment described herein and employing the RSV/anti-RSV system, the most beneficial of high affinity CDRs were found to result from amino acid replacements in 3 or 4 of the 6 CDRs, and in just 4 amino acid locations overall. Thus, the high affinity neutralizing antibodies of the present invention contain amino acid sequences differing from that of the
30 base or reference antibody only in complementarity determining regions, or in such regions as well as in surrounding framework regions, unlike previously known man-made antibodies. Thus, the antibodies of the present invention

are high affinity neutralizing antibodies containing one or more CDR sequences selected so as to produce high affinity in the antibody molecule. In the specific embodiment using RSV as the target such differences are found only in L2 (or CDRL2), L3 (or CDRL3), H1 (or CDRH1) and H3 (or CDRH3).

5 As noted, the greatest affinities for this antibody occurred only at selected amino acid positions of these CDRs, and in some of the embodiments of the present invention just one amino acid location in each CDR was preferred for giving high affinity.

10 Thus, for CDR H1, substitution at amino acid 2 of the CDR (counting from the amino terminal end of the particular underlined CDR sequence of Figure 1B), especially by replacing the serine located at position 2 of CDR H1 of the basic or reference antibody with either an alanine or a proline, was found to be most beneficial and therefore to result in higher affinity for the
15 RSV antigen epitope. For CDR H3, replacement of the glycine at position 6 of the CDR sequence, especially by either a phenylalanine or tryptophan, most especially by phenylalanine, was found to result in increased affinity for the RSV epitope. For CDR L2, replacement of the serine at position 3 of the CDR, especially by either a phenylalanine or a tyrosine, resulted in increased
20 affinity for F antigen. For CDR L3, replacement of the glycine at position 5 of the CDR, especially by phenylalanine, tryptophan or tyrosine, resulted in increased affinity for the RSV epitope.

In accordance with the invention, by combining such amino acid
25 substitutions so that more than one occurred in the same antibody molecule, it was possible to greatly increase the affinity of the antibodies disclosed herein for the epitope of the F antigen of RSV.

Table 2 shows the results of using the novel CDR sequences (for H1,
30 H3, L2, and L3, respectively) of a number of clones according to the present invention. As shown in Table 2, a particular antibody may have incorporated therein as many as 1, 2, or 3 novel CDRs of the invention versus the basic or

reference antibody chains shown in Figures 1A and 1B (for light (L2 and L3) and heavy (H1 and H3) chains, respectively). The effects of the novel CDRs are described in terms of Antigen (Ag) titration score (see below), where the basic or reference antibody is shown at the top and has a score of 0.1. Other scores are indicated relative to this 0.1 score of the "basic or reference antibody" sequences. The identities of the amino acids giving highest affinities at the respective locations (i.e., position 2 for H1, position 6 for H3, position 3 for L2, and position 5 for L3) are indicated below the amino acids for the basic or reference antibody merely to indicate the range of mutations used for each CDR.

The total number of clones examined in this experiment was 37, with some duplicates (indicated by the "n" value in parenthesis, for example, "n=4" for clone No. 7 indicates that 4 duplicate clones were examined).

In general, the data showed that there is a correlation between affinity and the number of beneficial or high affinity CDRs, with all of the higher affinity variants having more than one beneficial or high affinity CDR. Further, all of the best clones had an F (Phe) at position 6 within CDR H3. Also, the beneficial or high affinity CDRs were those wherein a hydrophobic, especially an aromatic, amino acid was inserted in place of the residue found in the basic or reference antibody.

The antibody titration assay employed varying concentrations of antibody using 500 ng of RSV F antigen for each measurement. A graph of comparison data for a number of the combinatorial clones of Table 2 is shown in Figure 2.

In sum, Table 2 shows a number of clones evaluated by the procedures described herein along with the amino acids occurring at the key locations (underlined and bold-faced in Tables 1 and 3) of CDRs H1, H3, L2, and L3. The Table also summarizes the number of differences between the

CDRs of these clones versus the corresponding CDRs of the reference antibody (See Table 1 and Figure 1). The right side of the Table shows an "Ag Score" or antigen binding value, which represents an arbitrary and qualitative value, ranging from 0 – 4, and represents a qualitative estimate of the relative binding ability of the different antibody clones based on their respective titration curves. This value is provided here only for rough qualitative comparisons of the different antibodies and is not intended as a quantitative measure of binding ability.

Table 2 also shows the number of novel CDRs for each antibody clone (meaning the number of CDRs in the antibody with at least one amino acid difference with respect to the corresponding CDR of the reference antibody – see Table 1). The number of novel CDRs is also the number of "beneficial" or "high affinity" CDRs present in that antibody molecule. The amino acid differences in the novel CDR would occur at the position bold and underlined in Table 1 for the reference antibody so that the amino acid bold and underlined in Table 1 has been replaced by the amino acid indicated for the respective CDR in Table 2 (using standard single letter amino acid designations).

Table 2. Summary of Clone Data

	Clone	CDRs				# Novel CDRs	Ag Score
		H1	H3	L2	L3		
5	Basic	S	W	S	G	0	0.1
	Single	A	F	F	F		
		P		Y	W		
					Y		
	1	<u>A</u>	<u>F</u>	<u>S</u>	<u>F</u>	3	4
10	2	<u>A</u>	<u>F</u>	<u>F</u>	G	3	4
	3 (n=3)	<u>P</u>	<u>F</u>	<u>F</u>	<u>F</u>	4	4
	4 (n=3)	<u>P</u>	<u>F</u>	<u>F</u>	<u>Y</u>	4	3.5
	5 (n=3)	<u>P</u>	<u>F</u>	<u>F</u>	<u>W</u>	4	3.5
	6	<u>P</u>	<u>F</u>	<u>Y</u>	<u>F</u>	4	3.5
15	7 (n=4)	<u>P</u>	<u>F</u>	<u>F</u>	G	3	3
	8	<u>P</u>	<u>F</u>	<u>F</u>	?	3+	3.5
	9 (n=2)	<u>P</u>	<u>F</u>	<u>S</u>	<u>W</u>	3	3
	10	<u>P</u>	<u>F</u>	<u>S</u>	<u>F</u>	3	3
	11	<u>P</u>	<u>W</u>	<u>F</u>	<u>W</u>	3	3
20	12 (n=2)	<u>P</u>	W	<u>F</u>	<u>F</u>	3	2.5
	13 (n=3)	S	<u>F</u>	<u>F</u>	<u>F</u>	3	2.5
	14	S	<u>F</u>	<u>F</u>	<u>W</u>	3	2.5
	15 (n=2)	<u>A</u>	<u>F</u>	S	G	2	2.5
	16 (n=2)	<u>P</u>	<u>F</u>	S	G	2	2
25	17	<u>P</u>	W	S	<u>W</u>	2	2
	18 (n=2)	S	<u>F</u>	<u>F</u>	G	2	2
	19	S	<u>F</u>	S	<u>W</u>	2	2
	20	S	<u>F</u>	S	<u>F</u>	2	2
	21	S	W	<u>Y</u>	<u>F</u>	2	2

30

The novel CDRs represented in each of the clones is readily determined by locating the clone in the table, and matching the indicated amino acid for each CDR with the corresponding amino acid for the same CDR next to the basic or reference clone. For convenience, where an amino acid is different in a particular CDR of one of the clones, the new amino acid is indicated in bold face. In addition, for all clones shown in the table, replacements occur only at the selected locations recited above as yielding a novel CDR. Thus, all substitutions in CDR H1 relative to the basic or reference antibody are at position 2 of the CDR (meaning, again, the second amino acid from the N-terminal end of CDR H1 as underlined and bold-faced in Figure 1 and Tables 1 and 3), all substitutions in CDR H3 are at position 6, all substitutions in CDR L2 are at position 3, and all substitutions in CDR L3 are at positions 5, again all with reference to the basic or reference antibody. It should be kept in mind that the basic or reference antibody was chosen because it was known already to have a very high affinity for RSV-epitopes. [See: Johnson et al, (1997) *J. Infect. Dis.*, **176**, 1215-1224] So, for example, the table shows that for clone No. 1, for the beneficial or high affinity CDR H1, an alanine is used in place of the serine at position 2 of CDR H1 of the basic or reference antibody, thereby achieving an increased affinity for RSV, and a phenylalanine occurs in place of the tryptophan at position 6 of CDR H3, the serine at position 3 of CDR L2 of the basic or reference antibody was used and a phenylalanine occurred at position 5 of beneficial or high affinity CDR L3.

Thus, the novel and beneficial CDRs according to the present invention (i.e., high affinity CDRs or CDR sequences whose presence in the basic or reference antibody in place of the corresponding basic or reference CDR served to greatly increase the affinity of said antibody for the same RSV epitope) which are present in the antibody structures produced in the supernatants tested for the clones of Table 2 are summarized in Table 3. In each case, the bold face indicates how the novel and beneficial, or high

affinity, CDRs of the invention differ from the corresponding CDRs of the basic or reference anti-RSV antibody.

5 Table 3. Sequences for High Affinity CDRs

	<u>CDR</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>
	H1	T <u>A</u> GMSVG	9
10	H1	T <u>P</u> GMSVG	10
	H3	SMITN <u>F</u> YFDV	11
	L2	DT <u>F</u> KLAS	12
15	L2	DT <u>Y</u> KLAS	13
	L3	FQGS <u>F</u> YPFT	14
	L3	FQGS <u>Y</u> YPFT	15
20	L3	FQGS <u>W</u> YPFT	16

While the CDR sequences of Table 3 represent the sequences for the high affinity CDRs disclosed according to the invention, it is understood that one or more of these CDRs may be present in the same antibody and the sequences of the table indicate the set from which appropriate sequences for each of the high affinity CDRs may be selected. Thus, as shown in Table 3, when a high affinity H1 CDR is present in a high affinity neutralizing antibody of the invention disclosed herein, it has a sequence corresponding to the sequence of SEQ ID NO: 9 or 10. When a neutralizing antibody of the claimed invention contains a high affinity H3 CDR, said CDR has the sequence of SEQ ID NO: 12. When a high affinity neutralizing antibody of the invention contains a high affinity L2 CDR, said high affinity L2 CDR has an amino acid

sequence selected from the group consisting of the sequences of SEQ ID NO:
12 and 13. Finally, when a high affinity neutralizing antibody of the present
invention contains a high affinity L3 CDR, said CDR has an amino acid
sequence selected from the group consisting of the sequences of SEQ ID NO:
5 14, 15 and 16.

As already stated, in one embodiment, the high affinity neutralizing
antibodies are antibodies that include a human constant region.

10 Thus, in a preferred embodiment, the high affinity neutralizing antibody
of the invention, with an affinity of at least 10^{10} M^{-1} , or even at least 10^{11} M^{-1} ,
is a grafted antibody that includes a human constant region and a framework
for the heavy and light chains wherein at least a portion of the framework is
derived from a human antibody (or from a consensus sequence of a human
15 antibody framework).

In another embodiment, all of the framework is derived from a human
antibody (or a human consensus sequence).

20 Thus, an RSV-neutralizing antibody, with an affinity of at least 10^{10} M^{-1} ,
is a grafted antibody having a human constant region, one or more CDRs that
are derived from a non-human antibody in which at least one of the amino
acids in at least one of said CDRs is changed and in which all or a portion of
the framework is derived from a human antibody (or a consensus sequence of
25 a human antibody framework).

Because the combination of CDR sequences of one antibody with non-
CDR regions of another antibody results from a form of "grafting" of CDRs
onto the remainder of the molecule, these have been referred to as "CDR
30 grafted" antibodies. Today, using the techniques of genetic engineering the
same product can be formed without isolating any sequences from actual
antibodies. So long as the desired CDR sequences, and the constant and

framework sequence are known, genes with the desired sequences can be assembled and, using a variety of vectors, inserted into appropriate cells for expression of the functional tetrameric antibody molecules. Coupling this with the methodology already described permits the assembly of single mutation
5 libraries wherein the antibodies possess the same sequences as corresponding grafted antibodies and, therefore, the same structure and binding affinities.

The high affinity antibodies of the invention can be present in a
10 relatively pure or isolated form as well as in a supernatant drawn from cells grown in wells or on plates. Such supernatants were used to generate the data of Table 1. The antibodies of the invention can thus also be present in the form of a composition comprising the antibody of the invention and wherein said antibody is suspended in a pharmacologically acceptable carrier,
15 or excipient. The antibodies of the invention may be present in such a composition at a concentration, or in an amount, sufficient to be of therapeutic or pharmacological value in treating diseases, such as RSV. Said antibodies may also be present in a composition in a more dilute form.

20 Consequently, the invention is also directed to providing a method of preventing and/or treating respiratory syncytial virus infections comprising the administering to a patient at risk thereof, or afflicted therewith, of a therapeutically (including prophylactically) effective amount of the antibody composition described herein.

25 One preferred embodiment of the high affinity antibodies of the present invention is the antibody whose heavy and light chain CDR regions have sequences as follows: CDR H1 has the sequence of SEQ ID NO: 9, CDR H3 has the sequence of SEQ ID NO: 11, CDR L2 has the sequence of SEQ ID NO: 4 (no change from CDR L2 of the reference sequence of Figure 1A), and
30 CDR L3 has the sequence of SEQ ID NO: 14 (see Table 3). In this preferred embodiment, the affinity constant is about 6.99×10^{10} (or about 14.3 pM as a dissociation constant) as shown in Table 4 (clone 1). The heavy and light

chain variable regions of an antibody comprising this embodiment, along with framework sequences, is shown in Figure 3.

Another preferred embodiment of the high affinity antibodies of the present invention is the antibody whose heavy and light chain CDR regions have the sequences as follows: CDR H1 has the sequence of SEQ ID NO: 9, CDR H3 has the sequence of SEQ ID NO: 11, CDR L2 has the sequence of SEQ ID NO: 12, and CDR L3 has the sequence of SEQ ID NO: 5 (see Table 2, clone 2 – no difference from the reference sequence of CDR L3 of Figure 1A). In this preferred embodiment, the affinity constant is about 7.30×10^{10} (or about 13.7 pM as a dissociation constant) as shown in Table 4 (clone 2). The heavy and light chain variable regions of an antibody comprising this embodiment, along with framework sequences, is shown in Figure 4.

An additional preferred embodiment of the high affinity antibodies of the present invention is the antibody whose heavy and light chain CDR regions have the sequences as follows: CDR H1 has the sequence of SEQ ID NO: 10, CDR H3 has the sequence of SEQ ID NO: 11, CDR L2 has the sequence of SEQ ID NO: 12, and CDR L3 has the sequence of SEQ ID NO: 14 (see Table 3 for CDR sequences) which clone is designated number 3 in Table 2. In this preferred embodiment, the affinity constant is about 8.13×10^{10} (or about 12.3 pM as a dissociation constant) as shown in Table 4 (clone 3). The heavy and light chain variable regions of an antibody comprising this embodiment, along with framework sequences, is shown in Figure 5.

A most preferred embodiment of the high affinity antibodies of the present invention is the antibody whose heavy and light chain CDR regions have the sequences as follows: CDR H1 has the sequence of SEQ ID NO: 9, CDR H3 has the sequence of SEQ ID NO: 11, CDR L2 has the sequence of SEQ ID NO: 12, and CDR L3 has the sequence of SEQ ID NO: 14 (see Table 3). In this preferred embodiment, the affinity constant is about 3.6×10^{11} (or about 2.8 pM as a dissociation constant) as shown in Table 4 (clone 22). The

heavy and light chain variable regions of an antibody comprising this embodiment, along with framework sequences, is shown in Figure 6.

Another most preferred embodiment of the present invention is the
5 antibody whose heavy and light chain CDR regions include the following
sequences: CDR H1 has the sequence of SEQ ID NO: 9, CDR H3 has the
sequence of SEQ ID NO: 11, CDR L2 has the sequence of SEQ ID NO: 12,
and CDR L3 has the sequence of SEQ ID NO: 15 (see Table 3). In this latter
preferred embodiment, the affinity constant is about 4×10^{11} (about 2.5 pM as
10 a dissociation constant) as shown in Table 4 (clone 23). The heavy and light
chain variable regions of an antibody comprising this embodiment, along with
framework sequences, is shown in Figure 7.

In particularly preferred embodiments, the antibodies of the present
15 invention will have the framework regions of the sequences depicted for the
framework regions shown in Figures 1, 3, 4, 5, 6, and 7 (each contains the
same framework regions and differ only in CDR sequences). These most
preferred embodiments include the neutralizing antibody wherein the light
chain variable region has the amino acid sequence of SEQ ID NO: 17 and the
20 heavy chain variable region has the amino acid sequence of SEQ ID NO: 18;
the neutralizing antibody wherein the light chain variable region has the amino
acid sequence of SEQ ID NO: 19 and the heavy chain variable region has the
amino acid sequence of SEQ ID NO: 20; the neutralizing antibody wherein the
light chain variable region has the amino acid sequence of SEQ ID NO: 21
25 and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 22; the neutralizing antibody wherein the light chain variable region has
the amino acid sequence of SEQ ID NO: 23 and the heavy chain variable
region has the amino acid sequence of SEQ ID NO: 24; the neutralizing
antibody wherein the light chain variable region has the amino acid sequence
30 of SEQ ID NO: 25 and the heavy chain variable region has the amino acid
sequence of SEQ ID NO: 26.

It should be kept in mind that while the high affinity neutralizing antibodies of the present invention can be assembled from CDR regions and non-CDR regions derived from actual neutralizing antibodies by splicing amino acid segments together (and antibodies so assembled would be within the invention disclosed herein) the antibodies of the present invention are most conveniently prepared by genetically engineering appropriate gene sequences into vectors that may then be transfected into suitable cell lines for eventual expression of the assembled antibody molecules by the engineered cells. In fact, such recombinant procedures were employed to prepare the antibodies disclosed herein. In addition, because the sequences of the chains of the high affinity antibodies are known from the disclosure herein, such antibodies could also be assembled by direct synthesis of the appropriate chains and then allowed to self-assemble into tetrameric (H₂L₂) bivalent antibody structures.

The method of preparing the high affinity neutralizing antibodies of the invention involved the creation of a combinatorial library which was used to prepare clones producing antibodies comprising the beneficial CDRs of the invention that could then be screened for affinity for RSV epitopes (for Example, Figure 2).

EXAMPLE 1

Kinetic Analysis of Humanized RSV Mabs by BIAcore™

The kinetics of interaction between high affinity anti-RSV Mabs and the RSV F protein was studied by surface plasmon resonance using a Pharmacia BIAcore™ biosensor. A recombinant baculovirus expressing a C-terminal truncated F protein provided an abundant source of antigen for kinetic studies. The supernatant, which contained the secreted F protein, was enriched approximately 20-fold by successive chromatography on concanavalin A and Q-sepharose columns. The pooled fractions were dialyzed against 10 mM

sodium citrate (pH 5.5), and concentrated to approximately 0.1 mg/ml. In a typical experiment, an aliquot of the F-protein (100 µl) was amine-coupled to the BIAcore sensor chip. The amount immobilized gave approximately 2000 response units (R_{max}) of signal when saturated with the mouse monoclonal antibodies H1129 or H1308F (prepared as in U.S. Patent 5,824,307, whose disclosure is hereby incorporated by reference). This indicated that there was an equal number of "A" and "C" antigenic sites on the F-protein preparation following the coupling procedure. Two unrelated irrelevant Mabs (RVFV 4D4 and CMV H758) showed no interaction with the immobilized F protein. A typical kinetic study involved the injection of 35 µl of Mab at varying concentrations (25-300 nM) in PBS buffer containing 0.05% Tween-20 (PBS/Tween). The flow rate was maintained at 5 ml/min, giving a 7 min binding phase. Following the injection of Mab, the flow was exchanged with PBS/Tween buffer for 30 min for determining the rate of dissociation. The sensor chip was regenerated between cycles with a 2 min pulse of 10 mM HCl. The regeneration step caused a minimal loss of binding capacity of the immobilized F-protein (4% loss per cycle). This small decrease did not change the calculated values of the rate constants for binding and dissociation (also called the k_{on} and k_{off} , respectively).

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More specifically, for measurement of k_{assoc} (or k_{on}), F protein was directly immobilized by the EDC/NHS method (EDC = N-ethyl-N'-[3-diethylamino-propyl]carbodiimide; NHS = N-hydroxysuccinimide) with the F-protein being injected over the EDC/NHS activated sensor chip. Briefly, 4 µg/ml of F protein in 10 mM NaOAc, pH 4.0 was prepared and about a 30 µl injection gives about 500 RU (response units) of immobilized F protein under the above referenced conditions. The blank flow cell (VnR immobilized-CM dextran surface) was subtracted for kinetic analysis. The column could be regenerated using 100 mM HCl (with 72 seconds of contact time being required for full regeneration). This treatment removed bound Fab completely without damaging the immobilized antigen and could be used for over 40 regenerations. For k_{on} measurements, Fab concentrations were 12.5 nM, 25

30

nM, 50 nM, 100 nM, 200 nM, and 400 nM. The dissociation phase was analyzed from 230 seconds (30 seconds after start of the dissociation phase) to 900 seconds. Kinetics were analyzed by 1:1 Langmuir fitting (global fitting). Measurements were done in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) Surfactant P20).

For measurements of combinatorial clones, as disclosed herein, the k_{on} and k_{off} were measured separately. The k_{on} was measured at conditions that were the same as those for the single mutation clones and was analyzed similarly.

For measuring k_{dissoc} (or k_{off}), the following conditions were employed. Briefly, 4100 RU of F protein were immobilized (as above) with CM-dextran used as the blank. Here, 3000 RU of Fab was bound (with dissociated Fab high enough to offset machine fluctuation). HBS plus 5 nM F protein (about 350 – 2000 times higher than the K_{dissoc} or K_d – the dissociation equilibrium constant) was used as buffer. The dissociation phase was 6 – 15 hours at a flow rate of 5 μ l/min. Under the conditions used herein, re-binding of the dissociated Fab was minimal. For further details, see the manual with the biosensor.

The binding of the high affinity anti-RSV antibodies to the F protein, or other epitopic sites on RSV, disclosed herein was calculated from the ratio of the first order rate constant for dissociation to the second order rate constant for binding or association ($K_d = k_{diss} / k_{assoc}$). The value for k_{assoc} was calculated based on the following rate equation:

$$dR/dt = k_{assoc}[Mab]R_{max} - (k_{assoc}[Mab] + k_{diss})R$$

where R and R_{max} are the response units at time t and infinity, respectively. A plot of dr/dt as a function of R gives a slope of $(k_{assoc} [Mab] + k_{diss})$ -- Since these slopes are linearly related to the [Mab], the value k_{assoc} can be derived from a

- replot of the slopes versus [Mab]. The slope of the new line is equal to k_{assoc} . Although the value of k_{dis} can be extrapolated from the Y-intercept, a more accurate value was determined by direct measurement of k_{diss} . Following the injection phase of the Mab, PBS/Tween buffer flows across the sensor chip.
- 5 From this point, [Mab]=0. The above stated equation for dR/dt thus reduces to:

$$dr/dt = k \quad \text{or} \quad dR/R = k_{\text{diss}} dt$$

- 10 Integration of this equation then gives:

$$\ln(R_0/R_t) = k_{\text{diss}} t$$

- where R_0/R_t are the response units at time 0 (start of dissociation phase) and t , respectively. Lastly, plotting $\ln(R_0/R_t)$ as a function of t gives a slope of k_{diss} .
- 15

In the preferred embodiment herein, the numerical values from such antibody variants were as follows:

20

Table 4. Summary of Kinetic Constants from Ultra-high Affinity Antibodies.

25	Clone ID	CDRs	k_{assoc} ($M^{-1}\text{sec}^{-1}$)	k_{diss} (sec^{-1})	K_a (M^{-1})
	1	AFSF	1.13×10^5	1.62×10^{-6}	6.98×10^{10}
	2	AFFG	1.33×10^5	1.82×10^{-6}	7.31×10^{10}
	3	PFFF	1.10×10^5	1.35×10^{-6}	8.15×10^{10}
30	22	AFFF	1.34×10^5	3.70×10^{-7}	3.62×10^{11}
	23	AFFY	1.22×10^5	3.03×10^{-7}	4.03×10^{11}

Here, the CDRs represent the amino acids replacing the reference amino acids at the key positions (or critical positions) of the CDRs shown in Table 1 (in bold and underlined) for a reference antibody. Thus, for example, clone 22 has an alanine at position 2 of CDR H1 (residue 32 of the heavy chain variable region – SEQ ID NO: 24) in place of the serine shown at that position in Table 1 (SEQ ID NO: 6), a phenylalanine at position 6 of CDR H3 (residue 105 of the heavy chain variable region – SEQ ID NO: 24) in place of the tryptophan shown at that position in Table 1 (SEQ ID NO: 8), a phenylalanine at position 3 of CDR L2 (residue 51 of the light chain variable region – SEQ ID NO: 23) in place of the serine shown at that position in Table 1 (SEQ ID NO: 4), and a phenylalanine at position 5 of CDR L3 (residue 92 of the light chain variable region – SEQ ID NO: 23) in place of the glycine shown at that position in Table 1 (SEQ ID NO: 5).

Of course, in forming such clones, Table 3 represents a pool of potential CDRs from which the high affinity CDRs of the antibodies of the present invention are to be drawn. For example, clone 23 of Table 4 uses the same CDRs as clone 22 with the exception of CDR L3, which has a tyrosine at position 5 of CDR L3 (Table 3 – SEQ ID NO: 15) in place of the glycine shown in that position in Table 1 (SEQ ID NO: 5). The substitutions at the corresponding critical positions are likewise shown in Table 2.

EXAMPLE 2

Microneutralization Assay

Neutralization of the antibodies of the present invention were determined by microneutralization assay. This microneutralization assay is a modification of the procedures described by Anderson et al (1985). Antibody dilutions were made in triplicate using a 96-well plate. Ten TCID₅₀ of respiratory syncytial virus (RSV – Long strain) were incubated with serial dilutions of the antibody (or Fabs) to be tested for 2 hours at 37°C in the wells

of a 96-well plate. RSV susceptible HEP-2 cells (2.5×10^4) were then added to each well and cultured for 5 days at 37°C in 5% CO₂. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F

5 protein expression. Fixed cells were incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The neutralizing titer was

10 expressed as the antibody concentration that caused at least 50% reduction in absorbency at 450 nm (the OD₄₅₀) from virus-only control cells. Results for several antibodies of the present invention are shown by the graph in Figure 8 while results using Fab fragments are depicted in the graph of Figure 9.

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WHAT IS CLAIMED IS:

1. A high affinity neutralizing immunoglobulin comprising at least three high affinity complementarity determining regions (CDRs) wherein each said
5 high affinity CDR has an amino acid sequence selected to result in an immunoglobulin with specificity toward at least one antigenic determinant and having an affinity constant (K_a) of at least $10^{10}M^{-1}$ for said antigenic determinant.
- 10 2. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin comprises at least four high affinity CDRs.
3. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin has 3 high affinity CDRs.
- 15 4. The high affinity neutralizing immunoglobulin of claim 2 wherein said immunoglobulin has 4 high affinity CDRs.
5. The high affinity neutralizing immunoglobulin of claim 2 wherein said
20 affinity constant (K_a) is at least 10^{11} .
6. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin is specific for at least one protein expressed by a virus.
- 25 7. The high affinity neutralizing immunoglobulin of claim 6 wherein said virus is respiratory syncytial virus (RSV).
8. The high affinity neutralizing immunoglobulin of claim 7 wherein the protein is the F protein of RSV.
- 30 9. The high affinity neutralizing immunoglobulin of claim 1 wherein the immunoglobulin has human constant regions.

10. The high affinity neutralizing immunoglobulin of claim 1 wherein the immunoglobulin binds to the same epitope on RSV as the reference immunoglobulin of Figure 1.

5

11. The high affinity neutralizing immunoglobulin of claim 1 wherein the immunoglobulin includes framework derived from only a human immunoglobulin.

10

12. The high affinity neutralizing immunoglobulin of claim 1 wherein at least a portion of the framework is derived from a murine immunoglobulin.

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13. The high affinity neutralizing immunoglobulin of claim 1 wherein at least one high affinity CDR contains a phenylalanine residue at a position where a non-phenylalanine residue occurs in the corresponding CDR of the basic or reference antibody of Figure 1.

20

14. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin comprises a high affinity H1 CDR and said CDR has an amino acid sequence selected from the group consisting of SEQ ID NO: 9 and 10.

25

15. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin comprises a high affinity H3 CDR having the amino acid sequence of SEQ ID NO: 11.

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16. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin comprises a high affinity L2 CDR and said CDR has an amino acid sequence selected from the group consisting of SEQ ID NO: 12 and 13.

17. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin has a high affinity L3 CDR having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 15 and 16.

5 18. The high affinity neutralizing immunoglobulin of claim 1 wherein the H1 CDR has the amino acid sequence of SEQ ID NO: 9, the H3 CDR has the amino acid sequence of SEQ ID NO: 11, the L2 CDR has the amino acid sequence of SEQ ID NO: 4 and the L3 CDR has the amino acid sequence of SEQ ID NO: 14.

10

19. The high affinity neutralizing immunoglobulin of claim 1 wherein the H1 CDR has the amino acid sequence of SEQ ID NO: 9, the H3 CDR has the amino acid sequence of SEQ ID NO: 11, the L2 CDR has the amino acid sequence of SEQ ID NO: 12 and the L3 CDR has the amino acid sequence of
15 SEQ ID NO: 5.

20. The high affinity neutralizing immunoglobulin of claim 2 wherein the H1 CDR has the amino acid sequence of SEQ ID NO: 10, the H3 CDR has the amino acid sequence of SEQ ID NO: 11, the L2 CDR has the amino acid
20 sequence of SEQ ID NO: 12 and the L3 CDR has the amino acid sequence of SEQ ID NO: 14.

21. The high affinity neutralizing immunoglobulin of claim 2 wherein the H1 CDR has the amino acid sequence of SEQ ID NO: 9, the H3 CDR has the
25 amino acid sequence of SEQ ID NO: 11, the L2 CDR has the amino acid sequence of SEQ ID NO: 12 and the L3 CDR has the amino acid sequence of SEQ ID NO: 14.

22. The high affinity neutralizing immunoglobulin of claim 2 wherein the
30 H1 CDR has the amino acid sequence of SEQ ID NO: 9, the H3 CDR has the amino acid sequence of SEQ ID NO: 11, the L2 CDR has the amino acid

sequence of SEQ ID NO: 12 and the L3 CDR has the amino acid sequence of SEQ ID NO: 15.

23. The high affinity neutralizing immunoglobulin of claim 1 wherein the
5 heavy chain variable region has the amino acid sequence of SEQ ID NO: 17
and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 18.

24. The high affinity neutralizing immunoglobulin of claim 1 wherein the
10 heavy chain variable region has the amino acid sequence of SEQ ID NO: 19
and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 20.

25. The high affinity neutralizing immunoglobulin of claim 2 wherein the
15 heavy chain variable region has the amino acid sequence of SEQ ID NO: 21
and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 22.

26. The high affinity neutralizing immunoglobulin of claim 2 wherein the
20 heavy chain variable region has the amino acid sequence of SEQ ID NO: 23
and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 24.

27. The high affinity neutralizing immunoglobulin of claim 2 wherein the
25 heavy chain variable region has the amino acid sequence of SEQ ID NO: 25
and the heavy chain variable region has the amino acid sequence of SEQ ID
NO: 26.

28. A recombinant high affinity neutralizing immunoglobulin having an
30 affinity constant of at least 10^{10} M^{-1} , wherein said immunoglobulin comprises a
human constant region and a heavy and light chain framework region at least
part of which is derived from human antibodies.

29. The recombinant high affinity neutralizing immunoglobulin of claim
30 wherein the heavy and light chain framework regions are derived from a
consensus sequence of human antibodies.

5 30. The recombinant high affinity neutralizing immunoglobulin of claim
30 wherein the affinity constant is at least 10^{11} M^{-1} .

31. The recombinant immunoglobulin of claim 30 wherein the heavy
and light chain framework regions are derived from a consensus sequence of
10 human antibodies.

32. A composition comprising the immunoglobulin of claim 1 wherein
said immunoglobulin is suspended in a pharmacologically acceptable carrier.

15 33. A composition comprising the immunoglobulin of claim 6 wherein
said immunoglobulin is suspended in a pharmacologically acceptable carrier.

34. A method of preventing and/or treating a disease comprising
administering to a patient at risk thereof, or afflicted therewith, a
20 therapeutically effective amount of the immunoglobulin composition of claim
32.

35. A method of preventing and/or treating a virus-induced disease
comprising administering to a patient at risk thereof, or afflicted therewith, a
25 therapeutically effective amount of the immunoglobulin composition of claim
33.

36. A method of preventing and/or treating respiratory syncytial virus
comprising administering to a patient at risk thereof, or afflicted therewith, a
30 therapeutically effective amount of the immunoglobulin of claim 7 suspended
in a pharmaceutically acceptable carrier.

37. The high affinity neutralizing immunoglobulin of claim 3 wherein said immunoglobulin is selected from the group consisting of Fab, F(ab)'₂, a heavy-light chain dimer, a heavy chain and a light chain.

5 38. The high affinity neutralizing immunoglobulin of claim 4 wherein said immunoglobulin is selected from the group consisting of Fab, F(ab)'₂, a heavy-light chain dimer, a heavy chain and a light chain.

10 39. The high affinity neutralizing immunoglobulin of claim 1 wherein said immunoglobulin is an antibody.

40. The high affinity neutralizing immunoglobulin of claim 2 wherein said immunoglobulin is an antibody.

15 41. The high affinity neutralizing immunoglobulin of claim 6 wherein said immunoglobulin is selected from the group consisting of Fab, F(ab)'₂, a heavy-light chain dimer, a heavy chain and a light chain.

20 42. The high affinity neutralizing immunoglobulin of claim 7 wherein said immunoglobulin is selected from the group consisting of Fab, F(ab)'₂, a heavy-light chain dimer, a heavy chain and a light chain.

25 43. The high affinity neutralizing immunoglobulin of claim 8 wherein said immunoglobulin is selected from the group consisting of Fab, F(ab)'₂, a heavy-light chain dimer, a heavy chain and a light chain.

30 44. A method of preventing and/or treating a disease comprising administering to a patient at risk thereof, or afflicted therewith, a therapeutically effective amount of the immunoglobulin of claim 37 or 38 suspended in a pharmaceutically acceptable carrier.

45. A method of preventing and/or treating a virus-induced disease comprising administering to a patient at risk thereof, or afflicted therewith, a therapeutically effective amount of the immunoglobulin of claim 41 suspended in a pharmaceutically acceptable carrier.

5

46. A method of preventing and/or treating respiratory syncytial virus comprising administering to a patient at risk thereof, or afflicted therewith, a therapeutically effective amount of the immunoglobulin of claim 42 or 43 suspended in a pharmaceutically acceptable carrier.

10

15

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25

30

Figure 1**A**

DIQMTQSPST LSASVGDRVT ITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSGYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNWFYDV W GQGTTVTVSS 120
CDR H3

Figure 2

Affinity Comparison of Beneficial Com4 Clones

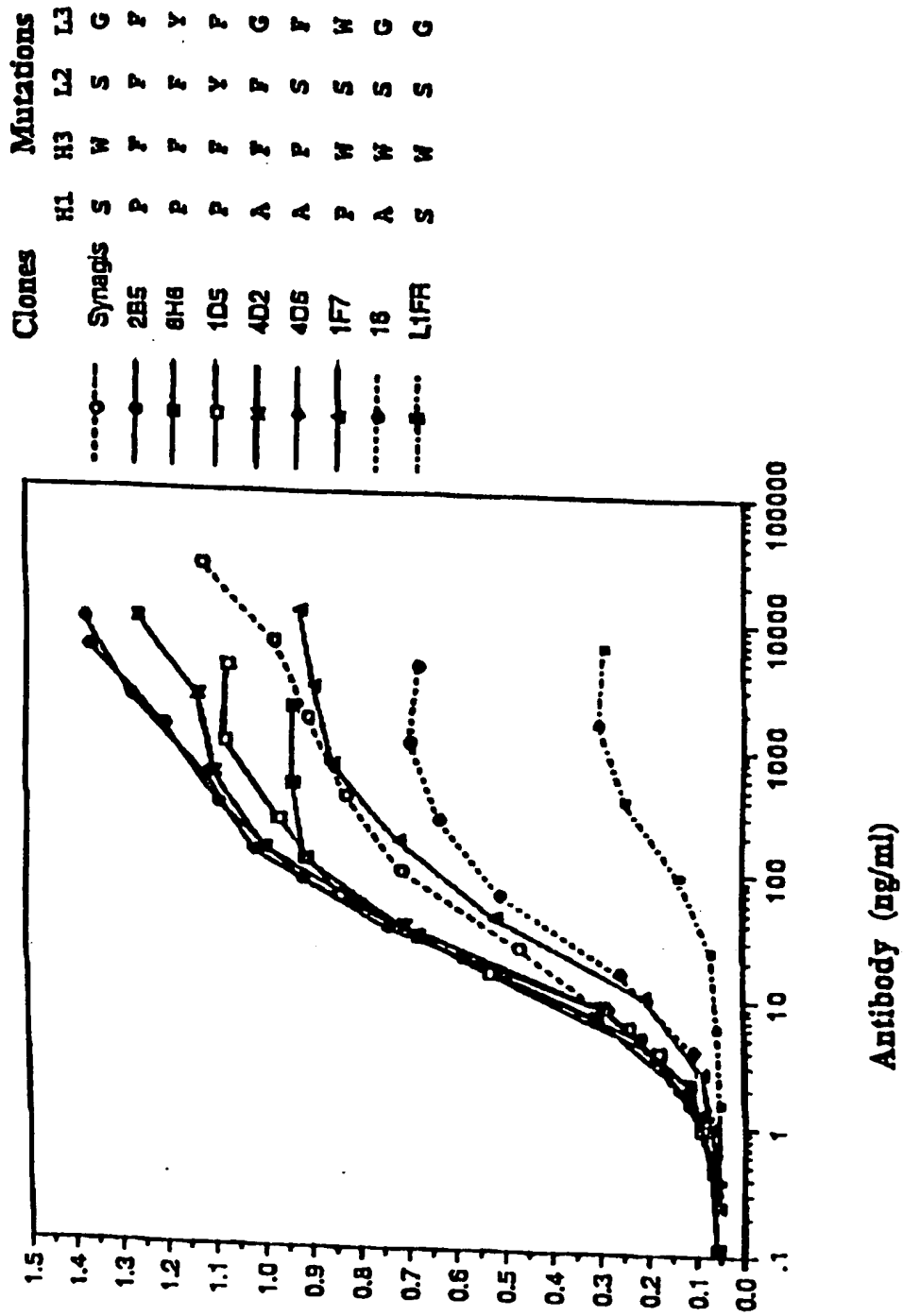


Figure 3**A**

DIQMTQSPST LSASVGDRVT ITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSFYPT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TAGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNFYFDV W GQGTTVTVSS 120
CDR H3

Figure 4**A**

DIQMTQSPST LSASVGDRVT ITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTFKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSGYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLs TAGMSVG WIR 40
CDR H1

QPRGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNFYFDV W GQGTTVTVSS 120
CDR H3

Figure 5**A**

DIQMTQSPST LSASVGDRVITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTFKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSFYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TPGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNFYFDV W GQGTTVTVSS 120
CDR H3

Figure 6**A**

DIQMTQSPST LSASVGDRVT ITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTFKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSFYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TAGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNFYFDV W GQGTTVTVSS 120
CDR H3

Figure 7**A**

DIQMTQSPST LSASVGDRVITC SASSSVGYMH WYQQKPG 40
CDR L1

KAPKLLIY DTFKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80
CDR L2

DFATYYC FQGSYYPFT FGGGTKVEIK 106
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TAGMSVG WIR 40
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80
CDR H2

VLKVTNMDPA DTATYYCAR SMITNFYFDV W GQGTTVTVSS 120
CDR H3

Figure 8

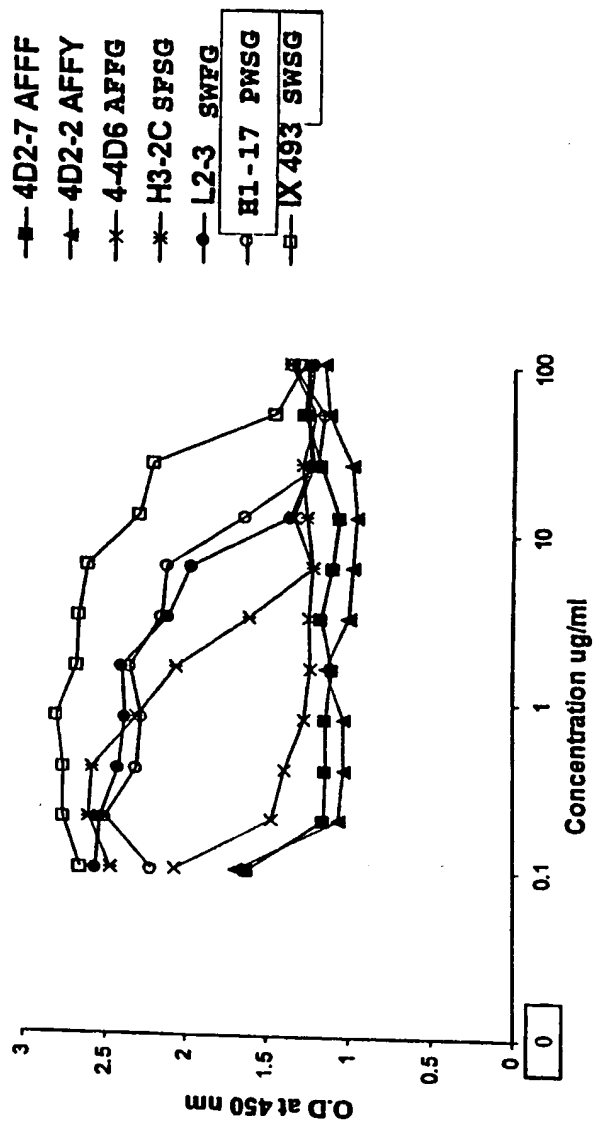


Figure 9

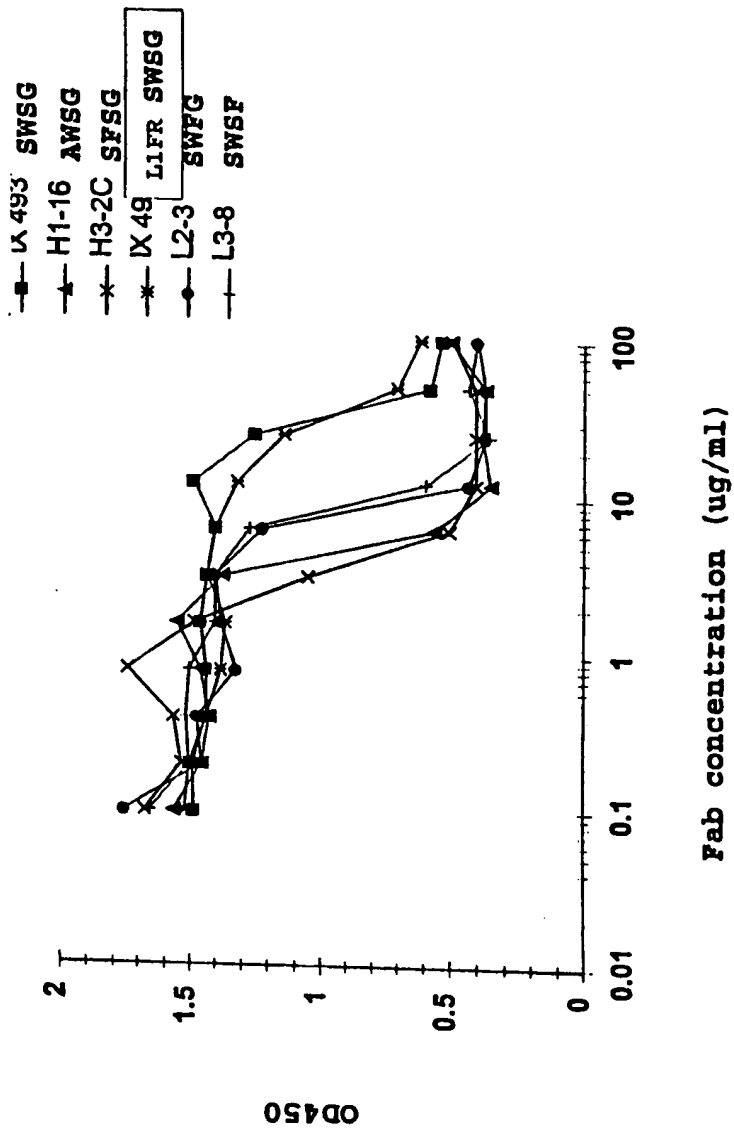
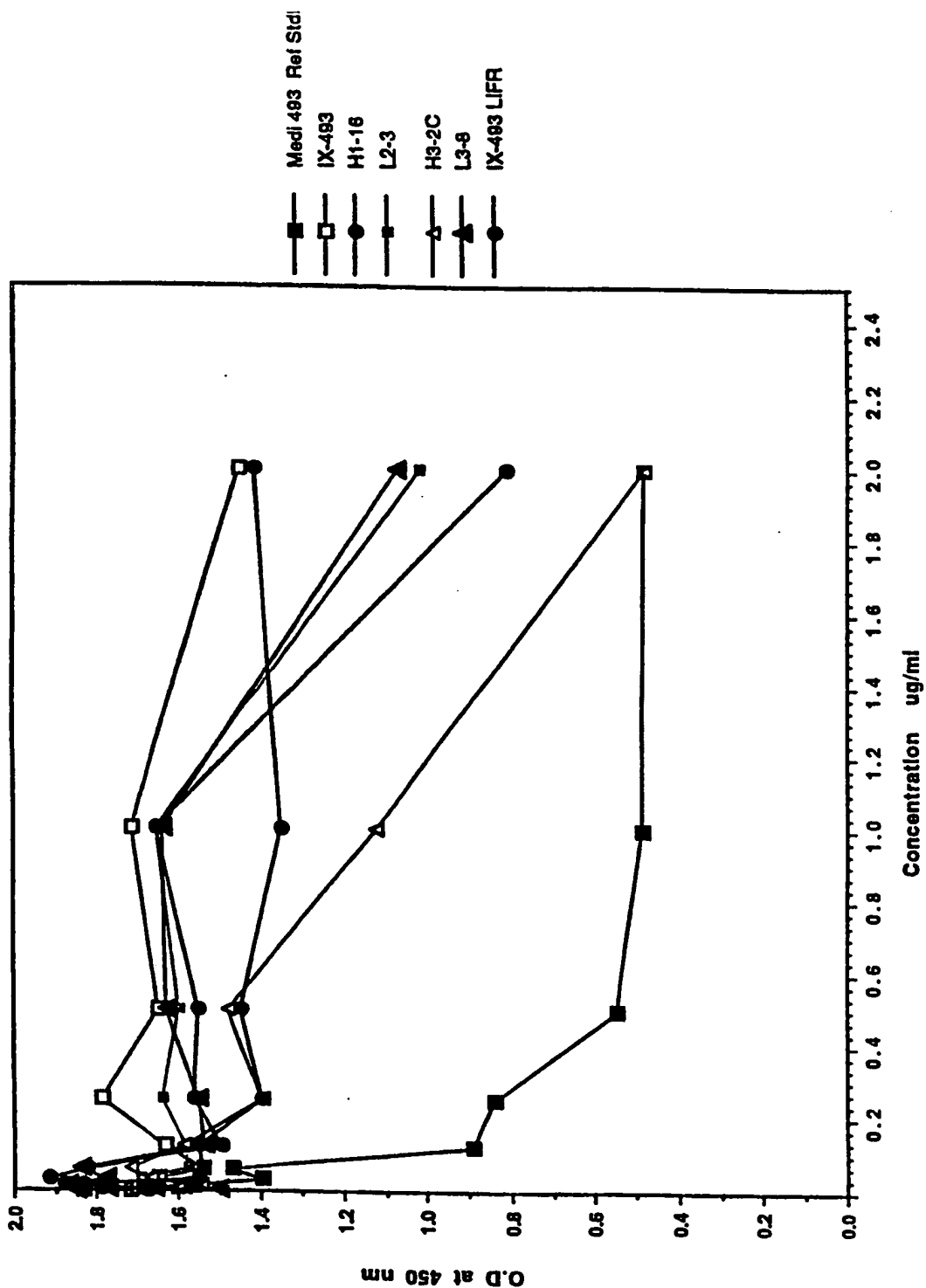


Figure 10

RSV Microneutralization Assay with Fab comparing to Med/493



SEQUENCE LISTING

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 Johnson, Leslie S.
 Huse, William D.
 Wu, Herren
 Watkins, Jeffry D.

<120> Ultra High Affinity Neutralizing Antibodies

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 35 40 45
 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
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chimeric antibody heavy chain variable chain

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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 3

<211> 10

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region L1
of reference anti-RSV antibody

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<210> 4

<211> 7

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region L2
of reference anti-RSV antibody

<400> 4

Asp Thr Ser Lys Leu Ala Ser
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<210> 5
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region L3
of reference anti-RSV antibody

<400> 5
Phe Gln Gly Ser Gly Tyr Pro Phe Thr
1 5

<210> 6
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region H1
of reference anti-RSV antibody

<400> 6
Thr Ser Gly Met Ser Val Gly
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<210> 7
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region H2
of reference anti-RSV antibody

<400> 7
Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser Leu Lys Ser
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<210> 8
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence of complementarity determining region H3
of reference anti-RSV antibody

<400> 8
Ser Met Ile Thr Asn Trp Tyr Phe Asp Val

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<210> 9

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 9

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<210> 10

<211> 7

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 10

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<210> 11

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 11

Ser Met Ile Thr Asn Phe Tyr Phe Asp Val

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<210> 12

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 12

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<210> 13
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 13
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<210> 14
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 14
Phe Gln Gly Ser Phe Tyr Pro Phe Thr
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<210> 15
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

<400> 15
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<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid
sequence present in high affinity complementarity
determining regions of antibodies of the invention

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Phe Gln Gly Ser Trp Tyr Pro Phe Thr
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<210> 17

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<212> PRT

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<223> Description of Artificial Sequence: Amino acid
 sequence of light chain variable region of clone 1
 of Figure 3A

<400> 17

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr
 85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 18

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Amino acid
 sequence of heavy chain variable region of clone 1
 of Figure 3B

<400> 18

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser

50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 19
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Amino acid
 sequence of light chain variable region of clone 2
 of Figure 4A

<400> 19
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
 20 25 30
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 35 40 45
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65 70 75 80
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
 85 90 95
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 20
 <211> 120
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 sequence of heavy chain variable region of clone 2
 of Figure 4B

<400> 20

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 21
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Amino acid
 sequence of light chain variable region of clone 3
 of Figure 5A

<400> 21
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
 20 25 30
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 35 40 45
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65 70 75 80
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr
 85 90 95
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 22

<211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Amino acid
 sequence of heavy chain variable region of clone 3
 of Figure 5B

<400> 22
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 23
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Amino acid
 sequence of light chain variable region of clone
 22 of Figure 6A

<400> 23
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
 20 25 30
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 35 40 45
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp

65						70						75				80
Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Phe	Gln	Gly	Ser	Phe	Tyr	Pro	Phe	Thr	
				85					90					95		
Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys							
			100					105								

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<210> 24
<211> 120
<212> PRT
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Amino acid
sequence of heavy chain variable region of clone
22 of Figure 6B

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<400> 24
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
  1              5              10              15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
      20              25              30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
      35              40              45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
  50              55              60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
  65              70              75              80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
      85              90              95

Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln
      100              105              110

Gly Thr Thr Val Thr Val Ser Ser
      115              120

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<210> 25
<211> 106
<212> PRT
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Amino acid
sequence of light chain variable region of clone
23 of Figure 7A

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<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
  1             5             10             15
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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
 20 25 30
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 35 40 45
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
 65 70 75 80
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Tyr Tyr Pro Phe Thr
 85 90 95
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 26

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Amino acid
 sequence of heavy chain variable region of clone
 23 of Figure 7B

<400> 26

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

INTERNATIONAL SEARCH REPORT

Inter. Appl. Application No

PCT/US 01/02618

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/10 A61K39/42 A61P31/12 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YANG W -P ET AL: "CDR WALKING MUTAGENESIS FOR THE AFFINITY MATURATION OF A POTENT HUMAN ANTI-HIV-1 ANTIBODY INTO THE PICOMOLAR RANGE" JOURNAL OF MOLECULAR BIOLOGY, GB, LONDON, vol. 254, 1995, pages 392-403, XP000199739 ISSN: 0022-2836 Discussion abstract</p> <p>---</p> <p>-/--</p>	<p>1-6,9, 11, 28-35, 37-41, 44,45</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

21 May 2001

Date of mailing of the international search report

06.06.01

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Renggli, J

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No

PCT/US 01/02618

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EGAN ROBERT W ET AL: "Effect of Sch 55700, a humanized monoclonal antibody to human interleukin-5, on eosinophilic responses and bronchial hyperreactivity." ARZNEIMITTEL-FORSCHUNG, vol. 49, no. 9, 1999, pages 779-790, XP000999038 ISSN: 0004-4172 abstract ---	1-5,9, 11-13, 28-32, 34, 37-40,44
X	WO 99 28471 A (US HEALTH ;PASTAN IRA H (US); CHOWDHURY PARTHA S (US)) 10 June 1999 (1999-06-10) claims 1-98; figure 1; example 4 page 21, line 22 -page 22, line 9 ---	1-5,9, 11-13, 28-32, 34, 37-40,44
X	US 5 929 212 A (ADAIR JOHN ROBERT ET AL) 27 July 1999 (1999-07-27) column 1, line 8 -column 1, line 28 seq ID:5, residues 71-77 column 6, line 34 -column 6, line 44 column 7, line 8 -column 7, line 11 figure 12 claims 1-13 ---	1-5,9, 11-13, 28-32, 34, 37-40,44
X	LOVE ROBERT A ET AL: "How the anti-(metal chelate) antibody CHA255 is specific for the metal ion of its antigen: X-ray structures for two Fab'/hapten complexes with different metals in the chelate." BIOCHEMISTRY, vol. 32, no. 41, 1993, pages 10950-10959, XP000999742 ISSN: 0006-2960 abstract; figure 5 ---	1-4,12, 13,32, 34, 37-40,44
X	WHITLOW MARC ET AL: "1.85 A structure of anti-fluorescein 4-4-20 Fab." PROTEIN ENGINEERING, vol. 8, no. 8, 1995, pages 749-761, XP000999039 ISSN: 0269-2139 Materials and methods abstract; figures 3-5 ---	1-4,12, 13,37-40
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/02618

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>WO 00 56771 A (HOLMES STEPHEN D ;SMITHKLINE BEECHAM PLC (GB); ABDEL MEGUID SHERIN) 28 September 2000 (2000-09-28)</p> <p>page 3, line 8 -page 4, line 30 page 6, line 31 -page 6, line 33 page 7, line 18 -page 9, line 15 page 10, line 14 -page 10, line 31 page 12 -page 20, line 12 page 20, line 29 -page 20, line 34 page 25; table 1 figures 1-6</p> <p style="text-align: center;">---</p>	<p>1-5,9, 11-13, 28-32, 34, 37-40,44</p>
P,X	<p>WO 00 73346 A (US HEALTH ;PASTAN IRA (US); CHOWDHURY PARTHA S (US)) 7 December 2000 (2000-12-07)</p> <p>page 2, line 27 -page 4 page 8, line 14 -page 8, line 19 page 12, line 14 -page 13, line 22 page 29, line 28 -page 30, line 14 claims 1-50 figures 1,4</p> <p style="text-align: center;">---</p>	<p>1-5,9, 11-13, 28-32, 34, 37-40,44</p>
A	<p>WO 96 05229 A (MEDIMMUNE INC) 22 February 1996 (1996-02-22)</p> <p>the whole document</p> <p style="text-align: center;">---</p>	<p>7,8,10, 12-27, 36,42, 43,46</p>
A	<p>BALINT R F ET AL: "ANTIBODY ENGINEERING BY PARSIMONIOUS MUTAGENESIS" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 137, no. 1, 27 December 1993 (1993-12-27), pages 109-118, XP002031537 ISSN: 0378-1119 page 110, right-hand column, line 7-11</p> <p style="text-align: center;">-----</p>	<p>7,8,10, 12-27, 36,42, 43,46</p>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/02618

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 34-36 and 44-46 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Appl. No.

PCT/US 01/02618

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 5929212 A	27-07-1999	AT 129017 T AT 124459 T AT 159299 T AU 664801 B AU 6461294 A AU 646009 B AU 6974091 A AU 649645 B AU 7033091 A AU 631481 B AU 7048691 A BG 60462 B BR 9007197 A CA 2037607 A CA 2046904 A CA 2050479 A,C DE 69020544 D DE 69020544 T DE 69022982 D DE 69022982 T DE 69031591 D DE 69031591 T DK 460167 T DK 460171 T DK 460178 T EP 0460167 A EP 0460171 A EP 0460178 A EP 0620276 A EP 0626390 A ES 2079638 T ES 2074701 T ES 2112270 T FI 990875 A WO 9109966 A WO 9109967 A WO 9109968 A GB 2246781 A,B GB 2246570 A,B GB 2268744 A,B GB 2268745 A,B GR 3017734 T GR 3025781 T HU 58372 A HU 215383 B HU 60786 A HU 58824 A,B	15-10-1995 15-07-1995 15-11-1997 30-11-1995 22-12-1994 03-02-1994 24-07-1991 02-06-1994 24-07-1991 26-11-1992 24-07-1991 28-04-1995 28-01-1992 07-09-1992 22-06-1991 22-06-1991 03-08-1995 18-01-1996 16-11-1995 28-03-1996 20-11-1997 12-03-1998 20-11-1995 28-08-1995 22-12-1997 11-12-1991 11-12-1991 11-12-1991 19-10-1994 30-11-1994 16-01-1996 16-09-1995 01-04-1998 19-04-1999 11-07-1991 11-07-1991 11-07-1991 12-02-1992 05-02-1992 19-01-1994 19-01-1994 31-01-1996 31-03-1998 28-02-1992 28-03-2000 28-10-1992 30-03-1992
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/02618

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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